Cells

Cell	Receptor
5.25	CXCR4, CD4, CCR5 (not expressed well) BONZO
5.25.Luc4.M7	CD4, CCR5, BONZO
HOS.CD4.CCR5	CD4, CCR5
HOS.CD4.CXCR4	CD4, CXCR4
HOS.CD4	CD4, low level expression of CCR5 and CXCR4
HOS HT4 R5 GFP wt	CD4, CXCR4, CCR5
HOS.CD4.CCR5.GFP.M7#6	6#CD4, CXCR4, CCR5
P4.CCR5	CD4, CXCR4, CCR5
₩2.CD.	CD4
U87.CD4 R5	CD4, CCR5
U87.CD4 X4 .	CD4, CXCR4
MT2	CD4, CXCR4
MT4	CD4, CXCR4
PM1	CD4, CXCR4, CCR5
CEM NKr CCR5	CD4, CXCR4, CCR5







${\tt Table 2}$ Representative viruses and reagents

Viruses	Envelope ^a	Source
89.6, SF2	R5-X4/SI/B	ARRRP ^B
92BR014,92US076	R5-X4/SI/B	ARRRP
JR-CSF, 91US005	R5/NSI/B	ARRRP
91US054	SI/B	ARRRP
NL43, MN, ELI	X4/B	ARRRP
92HT599	X4	ARRRP
92UG031	R5/NSI/A	ARRRP(IN-HOUSE)
92TH014, 92TH026	R5/NSI/B	ARRRP(IN-HOUSE)
92BR025, 93MW959	R5/SI/C	ARRRP(IN-HOUSE)
92UG035	R5/NSI/D	ARRRP(IN-HOUSE)
92TH022, 92TH023	R5/NSI/E	ARRRP(IN-HOUSE)
93BRU20	R5-X4/SI/F	ARRRP(IN-HOUSE)
Antipodies	Epitope	SOURCE
Mabs 2F5, 1577	gp41 TM	ARRRP
Mabs IG1b12, 2G12, 17b, 48D	gp120 SU	ARRRP
Neutralization sera #2, HIV-	Polyclonal	ARRRP
IG		
Entry inhibitors	Target	Source
CD4-IG	gp120 SU	Genentech
CD4-IGG2	gp120 SU	Adarc
SCD4	Sigma	Progenics
T20 (DP178)	gp41 TM	Trimeris
Rantes, MIP1a/b	CCR5	SIGMA/ARRRP
SDF1a/b	CXCR4	SIGMA/ARRRP
AMD 3100	CXCR4	AnorMed
Dextran sulfate, Heparin	Non-specific	Sigma

aR5 (CCR5 co-receptor), X4 (CXCR4 co-receptor)

SI (syncytium inducing), NSI (non-syncytium inducing), A,B,C,D,E,F (envelope clade designation)

bAIDS Research and Reference Reagent Program





Table 3

Primers Tested for the Amplification of HIV Envelope

RT PRIMERS

RT env_N3 RT env 9720

5'-GGA GCA TTT ACA AGC AGC AAC ACA GC-3'

RT env 9740

5' -TTC CAG TCA VAC CTC AGG TAC-3' 5'-AGA CCA ATG ACT TAY AAG G-3'

5' PCR PRIMERS

5'env

5' -GGG CTC GAG ACC GGT CAG TGG CAA TGA GAG TGA AG- 3'

5'env_Xho/Pin 5'-GGG CTC GAG ACC GGT GAG CAG AA-3 ACA GTG GCA ATG A-3' 5'env_START 5'-GGG CTC GAG ACC GGT GAG CAG AAG ACA GTG GCA ATG -3'

3' PCR PRIMERS

3' env

5' -GGG TCT AGA ACG CGT TGC CAC CCA TCT TAT AGC AA- 3'

3'env_Xba/Mlu 5'-GGG TCT AGA ACG CGT CCA CTT GC? ACC CAT BTT ATA GC-3' 5'-GGG TCT AGA ACG CGT CCA CTT GCC ACC CAT BTT A-3'

3'env_STOP 3' delta CT

| =

5' -GAT GGT CTA AGA CGC TGT TCA ATA TCC CTG CCT AAC TC- 3'

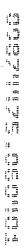
All Experiments are located in Virologic Book number 0188



Table 4 (Panel 1)

Anti-HIV Drugs

	Anu-	HIV Drugs	
Drug/Compound	Generic	Trademark	Manufacturer
	Name		
			Claus DA/s House
AZT, ZDV	Zidovudine	Retrovir	Glaxo/Wellcome .
3TC AZT + 3TC	Lamivudine	Epivir Combivir	Glaxo/Wellcome
	Standing	<u> </u>	Glaxo/Wellcome
d4T	Stavudine Didanosine	Zerit Videx	Bristol-Myers/Squibb
ddl ddC	Zalcitabine	Hivid	Bristol-Myers/Squibb Hoffman La Roche
1592U89		Ziagen	Glaxo/Wellcome
AZT + 3TC + 1592U89	Abacavir	Trizivir	Glaxo/Wellcome
(-)FTC (5-fluoro-3TC; Corviracil)	Emtricitabine	Trizivir	Triangle Pharmaceuticals
(-)FTC (5-11d070-31C, C07711acti)	Racimir	———	QuadPharma
DAPD (DXG active)	Amdoxovir		Triangle Pharmaceuticals
F-ddA (2-fluoro-ddA)	Lodenosine	· · · · · · · · · · · · · · · · · · ·	MedImmune Oncology (US Bioscience)
BCH-10652, dOTC	Lodellosille		BioChem Pharma, Inc.
(2-deoxy-3-oxa-4-thiocytidine)	·		Biochem Filanna, inc.
D-d4FC		<u> </u>	Triangle Pharmaceuticals (Schinazi)
RT inhibitors (NTRTI inucle of tide analo	(CS)		Thange Pharmaceuticals (Schillazi)
bis-POC PMPA (GS-4331)	Tenofovir		Gilead Sciences
bis-POM PMEA (GS-840)	Adefovir dipivoxil	 	Gilead Sciences
		l .	Gliead Sciences
BI-RG-587	Nevirapine	Viramune	Boehringer/Ingleheim (Roxanne)
BHAP PNU-90152T	Delavirdine	Rescriptor	Pharmacia & Upjohn
DMP 266 (L-743,726)	Efavirenz	Sustiva	Dupont Pharmaceuticals (Avid)
MKC442 (Coactinon)	Emivirine	Justiva	Triangle/Mitsubishi Kasei
AG-1549 (S1153) (on hold)	Capravirine		Agouron Pharmaceuticals
PNU-142721	Capiaviille		Pharmacia & Upjohn
DPC-961, -963, -083, -08?			DuPont Pharmaceuticals
SJ-3366	Also entry inhibitor?	· · · · · · · · · · · · · · · · · · ·	Samiin Pharmaceuticals
BHAP PNU-87201	Atevirdine		Upjohn
	(2 nd gen. HBY 097)		Glaxo/Wellcome (Hoechst Bayer)
GW420867X (quinoxaline)	(2 gen. HB1 097)		Tibotec
TMC 120 (R147681) TMC 125 (R165335)			Tibotec
R86183	tivironino		Janssen Pharmaceuticals
Calanolide A	tivirapine	 	Sarawak Medichem Pharmaceuticals
Drotogos Inhibitors (DDI)		1	Galawak Wedicherri Friamfaceuticals
Ro 31-8959	Saquinavir-(hgc) Saquinavir-(sgc)	Invirase Fortivase	Hoffman-La Roche
MK-639 (L-735,524)	Indinavir	Crixivan	Merck Research Laboratories
ABT-538 (A-84538)	Ritonavir	Norvir	Abbott Laboratories
AG1343	Nelfinavir	Viracept	Agouron Pharmaceuticals
141W94 (VX-478)	Amprenavir	Agenerase	Glaxo-Wellcome/Vertex
ABT-378/r	Lopinavir/ritonavir	Kaletra	Abbott Laboratories
BMS 232,632 (aza-peptide)	2001.121.1111.121.121.11		Bristol-Myers-Squibb
PNU-140690	Tipranavir		Pharmacia & Upjohn
DMP 450 (cyclic urea)	Mozenavir	****	Triangle/Avid (ph I/II)
TMC 126 (Erickson's compound)			Tibotec
G/W433908 (VX-175)	amprenavir pro-drug	 	Glaxo/Wellcome/Vertex
L756,423 (on hold)			Merck
PD-178390 (dihydropyrone)		1	Parke Davis (Boehringer-Ingleheim)
? new candidate		<u> </u>	Roche
DPC 681 and 684		1	DuPont Pharmaceuticals
AG-1776 (JE-2147 = KNI-764)		1	Agouron Pharmaceuticals
Envelope/Receptor Inhibitors			
T-20 (gp41)	Pentafuside		Trimeris Pharmaceuticals
T-1249 (gp41)	1	g .	Trimeris Pharmaceuticals
D-peptide inhibitor (gp41) small mol.	SCH-C	T	Schering-Plough
AMD-3100 (CXCR4)	(bicyclam)	T	AnorMED
AMD-8664 (CXCR4)	(macrocyclam)		AnorMED
ALX40-4C (CXCR4)		 	U. PA
FP21399			Fuji Pharmaceuticals
PRO 542 (gp120)	CD4lgG2	 	Progenics Pharmaceuticals
PRO 542 (gp120) PRO-140 (CCR5)	MAb CCR5		Progenics Pharmaceuticals Progenics Pharmaceuticals
	(peptide, 18-mer)	 	r regerinos i marmaceuticais
T-22 (CXCR4) Met-SDF-1 (CXCR4)	(peptide, 10-iller)		
			Takeda
TAK 779 (CCR5 antagonist)	 	-	Gryphon Sciences
AOP-Rantes (CCR5)	L	I	Gryphon aciences





SP1093V (BBNH Fe+3 derivative)

Table 4 (Panel 2

Rantes 9-68 (CCR5)			
CCR5 antagonists	4-(piperidin-1-yl) butane class		Merck
α-Immunokine-NNS03 (CCR5, CXCR4			PhyloMed Corp.
Integrase Inhibitors		T. S. 1828 17. 17. 18	STATE STATES OF STATES
AR-177	Zintevir		Aronex Pharmaceuticals
Diketo acids			Merck Research Laboratories
Nucleocapsid Inhibitors	11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Property of the Contract of th	To the second se
RB 2121	cyclic peptide p7 mimic	•	(see PNAS 96:4886-4891 (1999))
CI-1012			Achelion Pharmaceuticals
RNase Hilphibitor	100	515 THE 2008 THE STREET	497

FDA approved drugs are shown in boldface red = discontinued development, blue = not sure about development status

(Parniak)



Table 4 (Panel

Firm

Generic Name (abbreviation) zidovudine, AZT - didanosine, ddl zalcitabine, ddC stavudine, d4T lamivudine, 3TC saquinavir, SQV, hgc saquinavir, SQV, sgc ritonavir, RTV indinavir, IDV nevirapine, NVP nelfinavir, NFV delavirdine, DLV ZDV+3TC efavirenz, EFV abacavir, ABC amprenavir lopinavir/ritonavir ZDV+3TC+ABC

Brand Name Retrovir Videx Hivid Zerit **Epivir** Invirase Fortovase Norvir Crixivan Viramune Viracept Rescriptor Combivir Sustiva Ziagen Agenerase

Kaletra

Trizivir

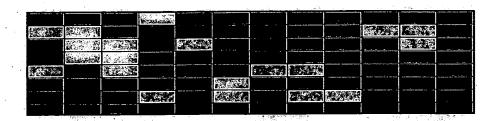
Glaxo Wellcome **Bristol Myers-Squibb** Hoffman-La Roche **Bristol Myers-Squibb** Glaxo Wellcome Hoffman-La Roche Hoffman-La Roche Abbott Laboratories Merck & Co., Inc. Boehringer Ingelheim Agouron Pharmaceuticals Pharmacia & Upjohn Glaxo Wellcome **DuPont Pharmaceuticals** Glaxo Wellcome Glaxo Wellcome Abbott GlaxoSmithKline

FDA Approval Date March 87 October 91 June 92 June 94 November 95 December 95 November 97 March 96 March 96 June 96 March 97 April 97 September 97 September 98 February 99 April 99 September 2000 November 2000

CORECEPTOR ASSAY SCREEN TEMPLATE

	RLU limit:	R ASS	4 Y SC	KEEN tropism ra		LAIE 5		CXCR4	CCR5	DUAL			drug 1: I drug 2: /	L83 AMD				
· ·	R5	1 X4	R5:X4	R5	2 X4	R5:X4	R5	3 X4	R5:X4	R5	4 X4	R5:X4	R5	5 X4	R5:X4	R5	6 X4	R5:X4
No Drug RLU L63 RLU AMD RLU	34	26,471 19,258 32	· 0.00	14,140 · 54 12,186	55	257.09	46	6,849 ; 4,696 40	0.01	21,656 . 27 . 7,226	38,144 25,542 32	0.57	30	7,336 5,468 39	0.00	3,988 34 3,664	31	128.6
No Drug RLU L83 RLU	R5 4,552 28	13 X4 19,102 9,956	R5:X4 0.24	R5 149 40	14 X4 226 153	R5:X4 0.66	R5 67,389 99	15 X4 72	R5:X4 935,96	R5 - 611 30	16 X4 19,715 14,985	R5:X4 0.03	· R5 46	17 X4 3,948 2,953	R5:X4 0.01	R5 284 38	18 X4 4,330 2,918	R5:X
AMD RLU No Drug RLU	838 R5 67,828	25 X4 68	R5:X4 997:47	R5 14,982	26 X4 12,020	R5:X4 1.25	42,295 R5 3,788	27 X4 4,384	R5:X4 0.86	76 • R5 50	1,022 28 X4 37	R5:X4	R5 658	152 29 X4 242	R5:X4	25 R5	30 X4 35	R5:X4
L83 RLU AMD RLU	129 51,146	37		111 8,580	10,839 3,384 38	٠.	59 2,049	3,397 538 39			40		32 493	221 38		47 219	42	
No Drug RLU L83 RLU AMD RLU	. R5	39 T	R5:X4	3,724 38 1,984	2,310 2,656 272	R5:X4 1.61	450 37 206	X4 668 463 72	R5:X4 0.67	R5 148 33 139	X4 24	R5:X4 , 6.17	R5 32	22	R5:X4	R5 60	84 61	R5:X4
No Drug RLU L83 RLU AMD RLU	R5 35	49 X4 107 68 34.	R5:X4 0.33	R5 83	3,502 88	R5:X4 20.02	R5 18,099 13,896 16,980	51 X4 8,451 6,220 8,711	R5:X4 2.14	R5 39,257 85 27,832	52 X4 38	R5:X4 1033:08	R5 5,413 35 5,043	53 X4 40	R5:X4 135.33	R5 97,733 30 48,452	54 X4 29	R5:X4
No Drug RLU L83 RLU AMD RLU	R5 3,769 29 2,773		R5:X4 ⊹94.23	R5 ,786 39 797		R5:X4 ∰ ≰16.38	R5 340 31 282		R5:X4 ≨316:19	R5 5,189 34 5,356	64 X4 43 T	R5:X4 120:67	R5 4,318 28 3,461	X4 37 [§]	R5:X4 ≈ 116.70	R5 196 23 184	X4 51	R5:X4 3.84
No Drug RLU L83 RLU AMD RLU	R5 391 50 340	73 X4 26	R5:X4 15.04	R5 98	74 X4 38	R5:X4	R5 4,449 . 43 3,752	·	R5:X4 :158.89	R5 4,357 34 906	76 X4 9,102 7,932 36	R5:X4 0.48	R5 6,090 73 4,473	77 X4 105 . 77 51	R5:X4 58.00	R5 1,886 38 279	3,635 2,092 27	R5:X4 0.52
No Drug RLU L83 RLU AMD RLU	R5 510 42 564	85 X4 33	R5:X4 (45:45	R5 979 55 813	86 X4 59	R5:X4 ⊹ 16:59	R5 491 49 376	87 X4 33	R5:X4 :14:88	R5 300 29 268	88 X4 3,815 ∂ 3,661 36	R5:X4 ∷% 0.08	R5 297 32 256	89 X4 3,615 2,946 30	R5:X4 	R5 39	90 X4 61,594 2 56,739 40	R5:X4 ↑∑≹ 0.00
	1	7			8			9	 1		- 10			11			12	
No Drug RLU L83 RLU AMD RLU	R5 30	X4 1,217 ⊈ 814 42	R5:X4 -%⊋0.02	R5 1	1,128 934 37	R5:X4	R5 42,206 90 33,192	. X4 . 70	R5:X4 7 602.94	R5 45	X4 1,159 ℃ 761 33	R5:X4 ∴%0.04	R5 44,589 60 30,510		R5:X4 459.68	R5 32	22,279 31	R5:X4
No Drug RLU L83 RLU AMD RLU	R5 100	19 X4 5,204 ∰ 3,058 34	R5:X4 	R5 11,299 22 8,232		R5:X4 217.29	R5 1,273 26 1,129	21 X4 20 \$	R5:X4 ∛ 63.65	R5 7,375 40 3,961	X4 10,856 6,455 2,283	R5:X4 0.68	R5 4,397 42 1,656	23 X4 7,130 3,874 1,285	R5:X4 0.62	R5 16,115 68 11,980	24 X4 50	R5:X4
No Drug RLU L83 RLU AMD RLU	R5 53	31 X4 974 #8 828 95	R5:X4	R5 177 32 361	32 X4 32	R5:X4 ≨5.53	R5 24 739 31 21 767		R5:X4 -> 749.67	R5 612 48 479	+34 √ X4 45 ∰	R5:X4 ./- 13.60	R5 2,997 22 925	35 X4 9,695 7,261 35	R5:X4 0.31	R5 416 28 597	36 X4 31	R5:X4 13.42
No Drug RLU L83 RLU AMD RLU	R5 45	43 X4 338 \$7 207 29	R5:X4	R5 714 31 366	•	R5:X4 □ 15.87	R5 11,029 18 8,390		R5:X4 5-612.72	R5 2,908 31 2,306	•	R5:X4 67.63	R5 13,997 73 12,139		R5:X4 	R5 24,377 59 16,045	48 X4 34	R5:X4 716.97
No Drug RLU L83 RLU AMD RLU	R5 9,254 43 2,219	55 X4 23,846 9,150 40	R5:X4 0.39	R5 5,249 38 784	56 X4 20,393 8,290 38	R5:X4 0.26	R5 470 37 310		R5:X4	R5 19,175 96 19,638	183 35	R5:X4 152:18	R5 513 35 500		R5:X4 16.55	R5 3,264 30 3,038	60 X4 28	R5:X4 116.57
No Drug RLU L83 RLU AMD RLU	R5 3,853 39 2,108		R5:X4 50.04	R5 411 22 253		R5:X4 ∰12.09	R5 7,857 42 4,595		R5:X4 ∂-≚191.63	R5 25,437 35 14,749		R5:X4 669.39	R5 17,443 40 13,708		R5:X4 ;387.62	R5 16,707 104 12,486	72 X4 38	R5:X4 439.66
No Drug RLU L83 RLU AMD RLU	R5 263 33 143		R5:X4 ≪ે6.92	R5 3,890 44 986	80 X4 9,577 6,140 45	R5:X4 0.41	R5 2,089 33 1,105	81 X4 1,698 1,224 160	R5:X4 1.23	R5 475 30 159	82 X4 43	R5:X4 -11:05	R5 8,475 30 6,862		R5:X4 ; 223.03	R5 4,107 28 2,571	84 X4 33	R5:X4 124.45
No Drug RLU L83 RLU AMD RLU	R5 443 33 451	91 X4 13,452 12,051 51	R5:X4 30.03	R5 34	92 X4 128,238 } 85,018 45	R5:X4	R5 22	93 X4 62 ■	R5:X4	R5 45	94 X4 . 33 ■	R5:X4	R5 43	95 X4 .42 ■	R5:X4	R5 46	96 X4 35	R5:X4

	a a more of the	des	<u> </u>	40.00
			21	
		R5	X4	R5:X4
	no drug			
	R5 inhibitor			
	X4 inhibitor			
%inhib	by R5 inhibitor			
%inhib	by X4 inhibitor			





		76	
	R5	X4	R5:X4
no drug	家14,982	£12,020 .	数2.711
R5 inhibitor	N 33111	10,839	等性 小龙
X4 inhibitor	8,580	3,384	
%inhib by R5 inhibitor	99	10	
%inhib by X4 inhibitor	43	72	

		95	
	R5	X4	R5:X4
no drug	43	42	
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor		ĺ	



Table &

	1	Repeat 1	End time 6:39:36 PM		End temp. 21.7							
0.5 CP	PS (C	PS)										
26	648	54	6970	37406	7158	38	1386	930	100	1184	112	30488
19	248	262	72	18972	3478	3946	5010	46	14	11004	7474	60
	106	9038				26	976	34	38	48	9736	32
	38 122	2000 2984		16 40		36 20	298 23344	52 15340	14 44	54 156	52 40	38 32
	36	52				40	62	32	38	22	44	28
	22	32				3802	46	11470	1958	42	42	24
	30	64	36	3846	3390	57858	12620	126186	68	34	38	36
Plate	2	Repeat 1	End time 6:41:51 PM									
0.5 CP	PS (C 34	PS)										
26	294	56	6728	38882	7514	24	1048	1326	40	1134	82	27748
	956	190				4714	5398	58	26	10708	6786	40
	30	15002		42		44	972	30	28	42	9654	30
	40 92	2620 5434					378 24348	38 25446	22 34	32 96	52 22	30 24
	44	44		46		62	92	36	44	54	46	48
	30	44				3468	30	7684	1438	44	34	42
	36	54	30	3784	3840	65330	14284	130290	56	32	46	34
Plate	3	Repeat 1	End time 6:44:06 PM		End temp. 21.6							
0.5 CP	PS (C 16	PS)										
18	590	18	4306	23902	5386	. 30	924	894	32	660	48	23382
	698	148				3142	3160	40	46	7616	2842	32
	30	10252				34	842	30	32	44	7616	34
	28	2396				66	172	32	22	28	. 36	26
	64 16	3784 28				24 30	6188 38	5702 38	34 38	120 44	34 62	24 34
	38	52				1798	62	7324	1076	50	42	32
	32	50		3824		53670	13088	104608	32	38	50	32
Plate	4	Repeat 1	End time 6:46:20 PM		End temp. 21.8							
0.5 CF												
	24	PS)										
	24		5086	27182	5550	26	704	974	32	862	44	21176
19		(PS) 46 158				26 2694	704 2956	974 26	32 20	862 5294	44 4906	21176 44
19	24 926 214 56	46 158 11426	42 3252	15882 82	3026 270	2694 52	2956 814	26 36	20 40	5294 64	4906 6906	44 32
19	24 926 214 56 32	46 158 11426 2916	42 3252 556	15882 82 42	3026 270 30	2694 52 64	2956 814 242	26 36 38	20 40 24	5294 64 36	4906 6906 42	44 32 40
19	926 214 56 32 72	46 158 11426 2916 3220	42 3252 556 7618	15882 82 42 36	3026 270 30 38	2694 52 64 44	2956 814 242 12112	26 36 38 10878	20 40 24 32	5294 64 36 246	4906 6906 42 32	44 32 40 42
19	24 926 214 56 32	46 158 11426 2916	42 3252 556 7618 22	15882 82 42 36 30	3026 270 30 38 28	2694 52 64	2956 814 242	26 36 38	20 40 24	5294 64 36	4906 6906 42	44 32 40
19	24 926 214 56 32 72 32	46 158 11426 2916 3220 48	42 3252 556 7618 22 40	15882 82 42 36 30 8844	3026 270 30 38 28 86	2694 52 64 44 24	2956 814 242 12112 136	26 36 38 10878 30	20 40 24 32 56	5294 64 36 246 34	4906 6906 42 32 24	44 32 40 42 24
19	24 926 214 56 32 72 32 32 32 22	46 158 11426 2916 3220 48 38 34	42 3252 556 7618 22 40	15882 82 42 36 30 8844 3498 Start temp	3026 270 30 38 28 86 2754	2694 52 64 44 24 2386 59808 BarCode	2956 814 242 12112 136 54	26 36 38 10878 30 4956	20 40 24 32 56 1372	5294 64 36 246 34 50	4906 6906 42 32 24 46	44 32 40 42 24 28
19 11	24 926 214 56 32 72 32 32 22	46 158 11426 2916 3220 48 38 34 Repeat	42 3252 556 7618 22 40 40	15882 82 42 36 30 8844 3498 Start temp	3026 270 30 38 28 86 2754	2694 52 64 44 24 2386 59808 BarCode	2956 814 242 12112 136 54	26 36 38 10878 30 4956	20 40 24 32 56 1372	5294 64 36 246 34 50	4906 6906 42 32 24 46	44 32 40 42 24 28
19 11 Plate	24 9926 214 56 32 72 32 32 22 5 S (C	46 158 11426 2916 3220 48 38 34 Repeat 1	42 3252 556 7618 22 40 40 End time 6:48:35 PM	15882 82 42 36 30 8844 3498 Start temp 21.9	3026 270 30 38 28 86 2754 End temp. 21.9	2694 52 64 44 24 2386 59808 BarCode N/A	2956 814 242 12112 136 54	26 36 38 10878 30 4956	20 40 24 32 56 1372	5294 64 36 246 34 50	4906 6906 42 32 24 46	44 32 40 42 24 28
19 11 Plate	24 926 214 56 32 72 32 32 32 22 5 S (C	46 158 11426 2916 3220 48 38 34 Repeat	42 3252 556 7618 22 40 40 End time 6:48:35 PM	15882 82 42 36 30 8844 3498 Start temp 21.9	3026 270 30 38 28 86 2754 End temp. 21.9	2694 52 64 44 24 2386 59808 BarCode	2956 814 242 12112 136 54 11014	26 36 38 10878 30 4956 65428	20 40 24 32 56 1372 34	5294 64 36 246 34 50 40	4906 6906 42 32 24 46 24	44 32 40 42 24 28 18
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19 11 Plate	24 926 56 32 72 32 32 22 5 5 (C 38 38 26 42 28 36	46 158 11426 2916 3220 48 38 34 Repeat 1 CPS)	42 3252 556 7618 22 40 40 End time 6:48:35 PM 40 32 420 88 11690 44	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 38 40	3026 270 30 38 28 86 2754 End temp. 21.9 38 190 36 34 44 58	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 32	2956 814 242 12112 136 54 11014	26 36 38 10878 30 4956 65428	20 40 24 32 56 1372 34 46 28 34 54 26 32	5294 64 36 246 34 50 40 30 2298 24 26 36 36	4906 6906 42 32 24 46 24 1290 40 30 46 38	44 32 40 42 24 28 18
19 11 Plate	24 926 214 56 32 72 32 22 25 5 (C 38 38 26 42 28 36 46 22 36	46 158 11426 2916 3220 48 34 Repeat 1 2PS) 28 20 3260 378 62 22 23 88	42 3252 556 7618 22 40 40 End time 6:48:35 PM 40 32 420 88 11690 44	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 38 40 40	3026 270 30 38 28 86 2754 End temp. 21.9 38 190 36 34 44 58 42 36	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 32 26 40 BarCode	2956 814 242 12112 136 54 11014 46 46 46 102 40 42 38 34	26 36 38 10878 30 4956 65428	20 40 24 32 56 1372 34 46 28 34 54 26 32 200	5294 64 36 246 34 50 40 30 2298 24 26 36 36 36	4906 6906 42 32 24 46 24 34 1290 40 30 46 38 34	22 24 28 18 22 24 28 18
19 11 Plate 0.5 CF	24 9926 214 56 32 32 32 22 5 5 (C 38 38 26 42 28 36 46 22 36	46 158 11426 2916 3220 48 34 Repeat 1 CPS) 28 20 3260 378 62 22 38 80 Repeat 1	42 3252 556 7618 22 40 40 End time 6:48:35 PM 40 32 420 88 11690 44 52 52	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 40 40 Start temp	3026 270 30 38 28 86 2754 End temp. 21.9 38 190 36 34 44 58 42 36	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 32 26 40 BarCode	2956 814 242 12112 136 54 11014 46 46 46 102 40 42 38 34	26 36 38 10878 30 4956 65428	20 40 24 32 56 1372 34 46 28 34 54 26 32 200	5294 64 36 246 34 50 40 30 2298 24 26 36 36 36	4906 6906 42 32 24 46 24 34 1290 40 30 46 38 34	22 24 28 18 22 24 28 18
Plate 0.5 CF	24 926 214 56 32 72 32 32 22 5 5 (C 38 38 26 42 28 36 42 23 36 6 6 6 6 7 8 8 8 8 8 8 8 8 8 8 8 8 8 8	46 158 11426 2916 3220 48 38 34 Repeat 1 2PS) 28 20 3260 378 62 22 22 38 80 Repeat 1	42 3252 556 7618 22 40 40 End time 6:48:35 PM 40 32 420 88 11690 44 52 52 End time 6:50:50 PM	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 38 40 40 Start temp 21.8	3026 270 38 28 86 2754 End temp. 21.9 38 190 36 34 44 58 42 36	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 26 40 BarCode N/A	2956 814 242 12112 136 54 11014 46 46 102 40 42 238 34 52	26 36 38 10878 30 4956 65428 30 24 26 42 42 30 50 50	20 40 24 32 56 1372 34 46 28 34 54 26 32 200 22	5294 64 36 246 34 50 40 30 2298 24 26 36 36 36 40	4906 6906 42 32 24 46 24 1290 40 30 46 38 34 42	22 24 28 18 22 24 28 18 22 26 24 48 42 12 36 36
Plate 0.5 CF	24 9926 214 56 32 72 32 32 22 5 5 (C 38 38 26 42 28 36 46 22 36	46 158 11426 2916 3220 48 34 Repeat 1 CPS) 28 20 3260 378 62 22 38 80 Repeat 1	42 3252 556 7618 22 40 40 End time 6:48:35 PM 40 32 420 88 11690 44 52 52 End time 6:50:50 PM	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 40 40 Start temp 21.8	3026 270 30 38 28 86 2754 End temp. 21.9 38 39 36 34 44 42 36 End temp. 21.9	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 32 26 40 BarCode	2956 814 242 12112 136 54 11014 46 46 46 102 40 42 38 34	26 36 38 10878 30 4956 65428	20 40 24 32 56 1372 34 46 28 34 54 26 32 200	5294 64 36 246 34 50 40 30 2298 24 26 36 36 36	4906 6906 42 32 24 46 24 34 1290 40 30 46 38 34	22 24 28 18 22 24 28 18
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Plate 0.5 CF	24 926 32 72 32 22 5 5 (C 38 38 26 42 28 36 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	46 158 11426 2916 3220 48 38 34 Repeat 1 CPS) 28 20 3260 378 62 22 38 80 Repeat 1	42 3252 556 7618 22 40 40 End time 6:48:35 PM 40 32 420 88 11690 44 52 End time 6:50:50 PM	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 40 40 Start temp 21.8	3026 2700 3038 2886 2754 End temp. 21.9 38 1900 36 344 44 58 42 36 End temp. 21.9	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 32 40 BarCode N/A	2956 814 242 12112 136 54 11014 46 46 46 102 40 42 38 34 52	26 36 38 10878 30 4966 65428 30 24 26 42 42 30 50 54	20 40 24 32 56 1372 34 46 28 34 54 26 32 200 22	5294 64 36 246 34 50 40 30 2298 24 26 36 36 36 40	4906 6906 42 32 24 46 624 34 1290 40 30 46 38 34 42	44 32 40 42 24 28 18 18 22 26 24 48 42 12 36 36 40 40 40 36 32 52
Plate 0.5 CF	24 926 32 72 32 32 22 5 5 (C 38 38 26 46 22 36 46 23 36 46 23 36 46 28 36 46 28 36 46 46 46 46 46 46 46 46 46 46 46 46 46	46 158 11426 2916 3220 48 38 34 Repeat 1 (PS) 28 20 3260 378 62 22 22 38 80 Repeat 1 1 (PS)	42 3252 40 40 End time 6:48:35 PM 40 32 420 88 11690 44 52 End time 6:50:50 PM 40 32 6:50:50 PM	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 38 40 40 V 21.8 34 1068 48 48 28 32 40	3026 270 38 28 86 2754 End temp. 21.9 38 190 36 34 44 58 42 36 End temp. 21.9	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 26 40 BarCode N/A	2956 814 242 12112 136 54 11014 46 102 40 42 38 34 52	26 36 38 10878 30 4956 65428 30 24 26 42 30 50 54	20 40 24 32 56 1372 34 46 28 34 54 26 32 200 22	5294 64 36 246 34 50 40 40 30 2298 24 26 36 36 36 40 40	4906 6906 42 32 24 46 24 1290 40 30 46 38 34 42 40 1280 30 26	22 24 28 18 22 24 48 24 48 42 12 36 36 36 32 32 36
19 11 Plate 0.5 CF	24 926 32 72 32 22 5 5 (C 38 38 26 42 28 36 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	46 158 11426 2916 3220 48 38 34 Repeat 1 CPS) 28 20 3260 378 62 22 38 80 Repeat 1	42 3252 556 7618 22 40 40 End time 6:48:35 PM 40 32 420 88 11690 44 52 52 End time 6:50:50 PM	15882 82 42 36 30 8844 3498 Start temp 21.9 30 976 50 32 38 40 40 Start temp 21.8	3026 270 38 28 86 2754 End temp. 21.9 38 190 36 34 44 45 8 42 36 .End temp. 21.9	2694 52 64 44 2386 59808 BarCode N/A 234 58 34 32 32 32 40 BarCode N/A	2956 814 242 12112 136 54 11014 46 46 46 102 40 42 38 34 52	26 36 38 10878 30 4966 65428 30 24 26 42 42 30 50 54	20 40 24 32 56 1372 34 46 28 34 54 26 32 200 22	5294 64 36 246 34 50 40 30 2298 24 26 36 36 36 40	4906 6906 42 32 24 46 24 1290 40 30 46 38 34 42 40 1280 30 26 38 36 38	44 32 40 42 24 28 18 18 22 26 24 48 42 12 36 36 40 40 40 36 32 52





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5	4 0.5 CPS (C 16 24 24 132 32 34 24	70 46 116 30 84 28	30 118 84 32 12036 42	21.9 26 34 34 28 94 44	26 26 26 34 26 38 24	N/A 40 40 48 40 34 28	34 34 46 44 40	26 38 28 24 26	26 30 12 38 48	50 38 26 86 32	36 36 46 36 30	56 28 58 36 150
38	4 0.5 CPS (C 16 24 24 132 32 34 24 62	70 46 116 30 84 28 24	30 118 84 32 12036 42 58	21.9 26 34 34 28 94 44	26 26 34 26 38 24 66	N/A 40 40 48 40 34 28 26	34 34 46 44 40 28	26 38 28 24 26 44	26 30 12 38 48 32	50 38 26 86 32 22	36 36 46 36 30 30	56 28 58 36 150 28
762 48 39888 88 40 30 36 8416 1262 4096 1598 12372 52058 8050 2524 60 450 168 32 340 22410 534 1046 512 106 2080 172 170 38 38 30 444 7478 2458 10024 15470 30 46 16428 25792 4240 45094 2092 630 334 17130 388 2730 2498 732 358 5044 3236 202 2124 292 4806 13736 13012 12386 290 30 3986 886 4584 240 110 940 1286 162 6768 2204 456 816 438 294 260 44 524 40 34 22 38 20 Plate Repeat End time Start temp.	4 0.5 CPS (C 16 24 24 132 32 34 24 62 44	70 46 116 30 84 28 24 80	30 118 84 32 12036 42 58 60 End time	21.9 26 34 34 28 94 44 32 28 Start temp.	26 26 26 34 26 38 24 66 40 End temp.	N/A 40 40 48 40 34 28 26 28 BarCode	34 34 46 44 40 28	26 38 28 24 26 44	26 30 12 38 48 32	50 38 26 86 32 22	36 36 46 36 30 30	56 28 58 36 150 28
762 48 39888 88 40 30 36 8416 1262 4096 1598 12372 52058 8050 2524 60 450 168 32 340 22410 534 1046 512 106 2080 172 170 38 38 30 444 7478 2458 10024 15470 30 46 16428 25792 4240 45094 2092 630 334 17130 388 2730 2498 732 358 5044 3236 202 2124 292 4806 13736 13012 12386 290 30 3986 886 4584 240 110 940 1286 162 6768 2204 456 816 438 294 260 44 524 40 34 22 38 20 Plate Repeat End time Start temp.	4 0.5 CPS (C 16 24 132 32 34 42 62 44 Plate 5	70 46 116 30 84 28 24 80 Repeat 1	30 118 84 32 12036 42 58 60 End time	21.9 26 34 34 28 94 44 32 28 Start temp.	26 26 26 34 26 38 24 66 40 End temp.	N/A 40 40 48 40 34 28 26 28 BarCode	34 34 46 44 40 28	26 38 28 24 26 44	26 30 12 38 48 32	50 38 26 86 32 22	36 36 46 36 30 30	56 28 58 36 150 28
52058 8050 2524 60 450 168 32 340 22410 534 1046 512 106 2080 172 170 38 38 30 444 7478 2458 10024 15470 30 46 16428 25792 4240 45094 2092 630 334 17130 388 2730 2498 732 358 5044 3236 202 2124 292 4806 13736 13012 12386 290 30 3986 886 4584 240 110 940 1286 162 6768 2204 456 816 438 294 260 44 524 40 34 22 38 20 Plate End time Start temp. End temp. BarCode 32 40 13240 38 7756 38 3368 24 40	4 0.5 CPS (C 16 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34	70 46 116 30 84 80 Repeat 1	30 118 84 32 12036 42 58 60 End time	21.9 26 34 34 28 94 44 32 28 Start temp.	26 26 34 26 38 24 66 40 End temp.	N/A 40 40 48 40 34 28 26 28 BarCode N/A	34 34 46 44 40 28 28	26 38 28 24 26 44 34	26 30 12 38 48 32 32	50 38 26 86 32 22 36	36 36 46 36 30 30 18	56 28 58 36 150 28 28
30	4 0.5 CPS (C 16 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34	70 46 116 30 84 80 80 Repeat 1 CPS)	30 118 84 32 12036 42 58 60 End time #######	21.9 26 34 34 28 94 44 32 28 Start temp. 22	26 26 34 26 38 24 66 40 End temp.	N/A 40 40 48 40 34 28 26 28 BarCode N/A	34 34 46 44 40 28 28	26 38 28 24 26 44 34	26 30 12 38 48 32 32 32	50 38 26 86 32 22 36	36 36 46 36 30 30 18	56 28 58 36 150 28 28
2498 732 358 5044 3236 202 2124 292 4806 13736 13012 12386 290 30 3986 886 4564 240 110 940 1266 162 6768 2204 456 816 438 294 260 44 524 40 34 22 38 20 Plate Repeat End time Start temp. End temp. BarCode Start temp. End temp. BarCode 32 40 13240 38 7756 38 3368 24 40 36836 48 30392 36 914 34 44702 64 50 20 24 8048 996 3826 1714 11588 50234 9110 1574 46 536 270 20 382 21124 424 804 682 200 1888 240	4 0.5 CPS (C 16 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058	70 46 116 30 84 80 80 Repeat 1 CPS)	30 118 84 32 12036 60 End time ########	21.9 26 34 34 28 94 44 32 28 Start temp. 22	26 26 34 26 38 24 66 40 End temp. 22	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168	34 34 46 44 40 28 28 44 36 32	26 38 28 24 26 44 34 40 8416 340	26 30 12 38 48 32 32 32	50 38 26 86 32 22 36	36 36 46 36 30 30 18	56 28 58 36 150 28 28 28
290 30 3986 886 4584 240 110 940 1286 162 6768 2204 456 816 438 294 260 44 524 40 34 22 38 20 Plate Repeat End time Start temp. End temp. BarCode 1 ####### 22 22.1 N/A 0.5 CPS (CPS) 32 40 13240 38 7756 38 3368 24 40 36836 48 30392 36 914 34 44702 64 50 20 24 8048 996 3826 1714 11588 50234 9110 1574 46 536 270 20 382 21124 424 804 682 200 1888 240 108 28 46 30 288 9302 2154 14254 16620 46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 16 12 14 132 32 32 34 44 Plate 5 0.5 CPS (C 34 38 762 52058 106	70 46 116 30 84 80 80 11132 48 80500 2080	300 1188 844 32 12036 60 End time ####### 26 39888 2524 172	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170	26 26 34 26 38 24 66 40 End temp. 22	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38	34 34 46 44 40 28 28 28	26 38 28 24 26 44 34 40 8416 340 444	26 30 12 38 48 32 32 32 29548 1262 22410 7478	50 38 26 86 32 22 36 40 409 534 2458	36 36 46 36 30 30 18 30628 1598 1046 10024	56 28 58 36 150 28 28 28
######## 22 22.1 N/A Plate Repeat End time Start temp. End temp. BarCode 6 1 ####### 22 22.1 N/A 0.5 CPS (CPS) 32 40 13240 38 7756 38 3368 24 40 36836 48 30392 36 914 34 44702 64 50 20 24 8048 996 3826 1714 11588 50234 9110 1574 46 536 270 20 382 21124 424 804 682 200 1888 240 108 28 46 30 288 9302 2154 14254 16620 46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 24 24 132 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058 106 30	70 46 116 300 84 28 24 80 Repeat 1 CPS)	30 118 84 32 12036 42 58 60 End time ####### 26 39888 2524 172 16428	21.9 26 34 34 28 94 44 32 28 Start temp. 22	26 26 26 34 26 38 24 60 40 End temp. 22	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094	34 34 46 44 40 28 28 44 36 32 30 2092	26 38 28 24 26 44 34 40 8416 340 444 630	26 30 12 38 48 32 32 32 29548 1262 22410 7478 334	50 38 26 86 32 22 36 40 4096 534 2458 17130	36 36 46 36 30 30 18 30628 1598 1046 10024 388	56 28 58 36 150 28 28 34 12372 512 15470 2730
6 1 ###### 22 22.1 N/A 0.5 CPS (CPS) 32 40 13240 38 7756 38 3368 24 40 36836 48 30392 36 914 34 44702 64 50 20 24 8048 996 3826 1714 11588 50234 9110 1574 46 536 270 20 382 21124 424 804 682 200 1888 240 108 28 46 30 288 9302 2154 14254 16620 46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058 106 30 2498	70 46 116 30 84 80 80 Repeat 1 CPS)	30 118 84 32 12036 42 58 60 End time ####### 26 39888 2524 172 16428 358	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044	26 26 34 26 38 24 66 40 End temp. 22 34 40 450 38 4240 3236	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124	26 38 28 24 26 44 34 40 8416 340 444 630 292	26 30 12 38 48 32 32 29548 1262 22410 7478 334 4806	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736	36 36 46 36 30 30 18 30628 1598 1046 10024 388 13012	56 28 58 36 150 28 28 28 34 12372 512 15470 2730 2738
32 40 13240 38 7756 38 3368 24 40 36836 48 30392 36 914 34 44702 64 50 20 24 8048 996 3826 1714 11558 50234 9110 1574 46 536 270 20 382 21124 424 804 682 200 1888 240 108 28 46 30 288 9302 2154 14254 16620 46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058 106 30 2498 220	70 46 116 30 84 80 80 80 20 20 80 46 732 30 30	300 1188 844 322 12036 60 End time ######## 266 39888 2524 172 16428 3588 3586	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886	26 26 34 26 38 24 66 40 End temp. 22 34 40 450 38 4240 326 4584	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 110	26 38 28 24 26 44 34 40 8416 340 444 630 292 940	26 30 12 38 48 32 32 32 29548 1262 22410 7478 334 4806 1286	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162	36 36 36 30 30 30 18 30628 1598 1046 10024 388 13012 6768	56 28 58 36 150 28 28 28 34 12372 512 15470 2730 12386 2204
914 34 44702 64 50 20 24 8048 996 3826 1714 11588 50234 9110 1574 46 536 270 20 382 21124 424 804 682 200 1888 240 108 28 46 30 288 9302 2154 14254 16620 46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 24 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058 106 30 2498 290 456	70 46 116 30 84 80 80 80 2080 46 7322 30 816 Repeat	300 118 84 32 12036 60 End time ####### 266 39888 2524 172 16428 3588 3986 438 End time	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886 294 Start temp.	26 26 34 26 38 24 66 40 End temp. 22 34 40 450 38 4240 3236 4584 260	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 110	26 38 28 24 26 44 34 40 8416 340 444 630 292 940	26 30 12 38 48 32 32 32 29548 1262 22410 7478 334 4806 1286	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162	36 36 36 30 30 30 18 30628 1598 1046 10024 388 13012 6768	56 28 58 36 150 28 28 28 34 12372 512 15470 2730 12386 2204
914 34 44702 64 50 20 24 8048 996 3826 1714 11588 50234 9110 1574 46 536 270 20 382 21124 424 804 682 200 1888 240 108 28 46 30 288 9302 2154 14254 16620 46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058 106 30 2498 290 456 Plate 6 0.5 CPS (C	70 46 116 30 84 80 80 Repeat 1 CPS) 11132 48 8050 2080 46 7322 30 816 Repeat 1 CPS)	300 118 84 32 12036 60 End time ####### 266 39888 2524 172 16428 3588 3986 438 End time	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886 294 Start temp.	26 26 34 26 38 24 66 40 End temp. 22 34 40 450 38 4240 3236 4584 260	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 110	26 38 28 24 26 44 34 40 8416 340 444 630 292 940	26 30 12 38 48 32 32 32 29548 1262 22410 7478 334 4806 1286	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162	36 36 36 30 30 30 18 30628 1598 1046 10024 388 13012 6768	56 28 58 36 150 28 28 28 34 12372 512 15470 2730 12386 2204
200 1888 240 108 28 46 30 288 9302 2154 14254 16620 46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 24 132 32 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058 106 30 2498 2290 456 Plate 6 0.5 CPS (C	70 46 116 30 84 28 24 80 8 Repeat 1 CPS)	30 118 84 32 12036 42 12036 60 End time ######## 26 39888 2524 172 16428 358 3986 438 End time ####################################	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886 294 Start temp.	26 26 34 24 66 40 End temp. 22 34 40 450 3236 4584 260 End temp.	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode N/A	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 110 524	26 38 28 24 26 44 34 34 40 8416 340 444 630 292 940 40	26 30 12 38 48 32 32 29548 1262 22410 7478 334 4806 1286 34	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162 22	36 36 46 36 30 30 18 30628 1598 1046 10024 388 13012 6768 38	56 28 36 150 28 28 28 34 12372 512 15470 2730 12386 2204 20
46 28 17532 29872 5846 51810 2346 938 286 22146 612 3346 3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 16 24 24 132 34 24 62 44 Plate 5 0.5 CPS (C 34 38 762 52058 1066 30 2498 290 456 Plate 6 0.5 CPS (C 34 40 914 40 914	1 1 2 PS) 70 46 116 136 24 80 80 80 14 80 80 90 80 80 80 80 80 80 80 80 80 80 80 80 80	30 118 84 32 12036 42 58 60 End time ####### 26 39888 2524 172 16428 3986 438 End time #######	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 Start temp. 22	26 26 26 34 26 38 24 66 40 End temp. 22 34 40 450 38 4240 3236 4584 4584 260 End temp. 22.1	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode N/A	34 34 46 44 40 28 28 28 44 36 32 20 2092 2124 110 524	26 38 28 24 26 44 34 34 40 8416 340 444 630 292 940 40	26 30 12 38 48 32 32 32 29548 1262 22410 7478 334 4806 1286 34	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162 22	36 36 46 36 30 30 18 30628 1598 1046 10024 388 13012 6768 38	56 28 36 150 28 28 28 34 12372 512 15470 2730 12386 2204 20
3048 862 206 5668 3686 166 2092 214 4384 15762 14404 12586 390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 16 24 24 132 32 34 24 62 44 Plate 5 5 0.5 CPS (C 34 250 456 Plate 6 6 0.5 CPS (C 32 40 914 50234	70 46 116 30 84 28 24 80 82 24 80 80 20 80 20 80 46 732 30 816 Repeat 1 CPS)	######################################	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886 294 Start temp. 22	26 26 34 24 66 38 24 40 450 33 4240 3236 4584 260 End temp. 22.1	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode N/A 3368 20 270	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 110 524	26 38 28 24 26 44 34 34 40 8416 340 444 630 292 940 40 8048 382	26 30 12 38 48 32 32 29548 1262 22410 7478 334 4806 1286 34	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162 22	36 36 36 30 30 30 18 30628 1598 1046 10024 388 13012 6768 38	56 28 58 36 150 28 28 28 34 12372 512 15470 2730 12386 2204 20
390 64 3518 926 4362 318 176 1032 924 156 6956 2938	4 0.5 CPS (C 34 34 38 762 52058 106 30 456 Plate 6 0.5 CPS (C 32 44 50234 200 456 200 6 10 6 10 6 10 6 10 6 10 6 10 6 10	1 1 2 PS) 70 46 116 30 84 48 28 24 80 Repeat 1 2 PS) 11132 48 8050 20800 46 732 30 816 Repeat 1 2 PS)	######################################	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886 294 Start temp. 22 7756 64 64 64 108	22 26 26 34 26 38 24 66 40 22 22 34 40 450 38 4240 3236 4584 260 End temp. 22.1	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode N/A 3368 20 270 46	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 1110 524	26 38 28 24 26 44 34 34 40 8416 340 444 630 292 940 40 8048 382 288	26 30 12 38 48 32 32 29548 1262 22410 7478 334 4806 1286 34	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162 22	36 36 36 36 30 30 18 30628 1598 1046 10024 328 338 3012 6768 38	56 28 58 36 150 28 28 28 34 12372 512 15470 2730 12386 2204 20
	4 0.5 CPS (C 16 16 24 24 132 34 24 62 44 Plate 5 5 0.5 CPS (C 34 38 762 52058 1066 30 2498 2900 456 Plate 6 0.5 CPS (C 32 40 914 50234 200 466	10PS) 70 46 116 136 138 24 80 Repeat 1 10PS) 11132 48 8050 2080 46 732 30 816 Repeat 1 10PS) 13240 34 9110 1888 28	######################################	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886 294 Start temp. 22	22 26 26 34 26 38 24 66 40 End temp. 22 334 4240 3336 4584 260 End temp. 22.1	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode N/A 3368 20 270 46 51810	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 110 524	26 38 28 24 26 44 34 40 8416 340 444 630 292 940 40 8048 382 288 938	26 30 12 38 48 32 32 32 29548 1262 22410 7478 334 4806 1286 34 36836 996 21124 9302 286	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162 22	36 36 36 30 30 30 18 30628 1598 1046 10024 388 13012 6768 38	56 28 36 150 28 28 28 34 12372 512 15470 2730 12386 2204 20
	4 0.5 CPS (C 16 24 24 132 32 34 24 62 44 Plate 5 5 0.5 CPS (C 34 250 250 86 106 30 2498 220 456 Plate 6 6 0.5 CPS (C 32 40 914 50234 200 46 3048 3048 3048 3048 3048 3048 3048 3048	10 PS) 70 46 116 30 84 28 24 80 Repeat 1 CPS) 11132 48 8050 2080 46 732 30 816 Repeat 1 CPS)	######################################	21.9 26 34 34 28 94 44 32 28 Start temp. 22 6696 88 60 170 25792 5044 886 2947 Start temp. 22	26 26 26 34 24 66 40 22 22 34 40 450 3236 4584 260 50 536 28 5366 3886	N/A 40 40 48 40 34 28 26 28 BarCode N/A 3960 30 168 38 45094 202 240 44 BarCode N/A 3368 20 270 46 51810 166	34 34 46 44 40 28 28 28 44 36 32 30 2092 2124 110 524	26 38 28 24 26 44 34 34 40 8416 340 444 630 292 940 40 8048 382 288 938 938 214	26 30 12 38 48 32 32 29548 1262 22410 7478 334 4806 1286 34 36836 996 21124 9302 286 4384	50 38 26 86 32 22 36 40 4096 534 2458 17130 13736 162 22 22	36 36 36 36 30 30 18 30628 1598 1046 10024 388 13012 6768 38	56 28 58 36 150 28 28 28 34 12372 512 15470 2730 12386 2204 20 346 11588 682 16620 3346 12586 2938

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	29,118	20	31	8	28	88	33	32		22,279	38	33	335	33	82	90	52	-		31	33	စ္က	40	47	24	36	27
	97	7,130	9,695	25	31	45	38	45		46	3,874	7,261	33	33	43	4	37			37	1,285	35	78	45	35	27	4
	1,159	10,856	45	43	126	38	43	33		761	6,455	2	35	183	33	20	33			33	2,283	4	8	35	88	98	33
	2	8	33	18	89	41	1,698	62		32	8	36	23	33	47	1,224	33			9	53	33	43	38	37	160	11
	1,128	25	32	45	20,393	æ	9,577	128,238		934	33	33	32	8,290	¥	6,140	85,018			37	35	78	4	88	88	5	42
	1,217	5,204	974	338	3,846	77	38	13,452		814	3,058	828	202	9,150	87	28	12,051			42	¥	92	53	4	怒	33	51
					3					78	2,918	43	65	8	27	2,092	. 66,739			142	48	8	36	33	92	27	4
	7,336							_									2,946 5			33	152	38	56	43	4	51	30
								3,815									3,661			35	1,022	49	93	32	စ္တ	38	36
	6,849	72	4,384	899	8,451	21	78	33		4,696	38	3,397	463	6,220	42	4	55			9	35	538	72	8,711	4	33	22
	22	526	12,020	2,310	4,209	84	88	29		35	153	10,839	2,656	3,502	38	45	42			78	54	3,384	272	88	22	31	69
X4 No Drug	6,471	9,102	89	33	107	4	56	83		9,258	9,956	43	8	89	54	32	27		_	35	发	33	78	发	41	8	8
× %									L83					30					AMD	35	88	97	45	88	98	7	53
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	-				5 513															_		_			_	9 6,862	
	4	7,37	613	2,90	19 175	25,43	47	4						96							_		_	Ξ		159	
	4		.,	_	470									37						33,192	1,129	21,767	8,390	310	4,595	1,10	27
	49	11,299	177	714	5,249	411	3,890	34						88						4	8,232	361	366	784	253	986	35
	8	5	53	45	9,254	3,853	263	443		27	35	28	32	43	39	33	33			33	၉	56	9	2,219	2,108	143	451
	3,988	284	231	8	97,733	196	1,886	33		8	38	47	30	39	23	38	25			3,664	52	219	42	48,452	<u>\$</u>	279	35
	8	46	658	35	5,413	4,318	060'9	297		32	7	32	3	35	28	73	32			36	45	493	8	5,043	3,461	4,473	256
	21,656	611	22	148	39,257	5,189	4,357	300		27	8	38	33	82	¥	8	53			7,226	9/	23	139	27,832	5,356	906	268
	46	37,389	3,788	420	18,099	340	4,449	491		53	66	28	37	13,896	33	43	49			32	42,295	2,049	506	16,980	282	3,752	376
		_			8									•			22				_	_	_	Ĺ			813
RS No Drug	8		•						L83	90	78	129	53	31	83	20	42		_	Ξ	_		_	_	_	_	264



T20 Resistance Mutations

SDM Virus	T20 Sens.a	T20 FCb
HXB2 G I V		1.0
NL4-3 G I V	S	5.2
NL4-3 D I V	S	12.8
NL4-3 G I M	S	33.0
NL4-3 S I V	S	74.2
NL4-3 D I M	R	113.0
NL4-3 S I M	R	227.4
NL4-3 D T V	R	>281.8
JRCSF G I V		2.1
JRCSF D I V		104.0

^a Rimsky et al., J. Virol. 72(2):986-993

b Fold change in IC50 (vs. HXB2) using PhenoSense HIV Entry Assay





T20 Resistance Mutations

SDM Virus	<u>T20 S</u>	Sens.a	<u>T20_FC</u> b	
HXB2 G I V			1.0	
NL4-3 G I V		S	5.2	
NL4-3 D I V		S	12.8	
NL4-3 G I M	9	S	33.0	
NL4-3 S I V		Š	74.2	
NL4-3 D I M	. 1	R	113.0	
NL4-3 S I M	·	R .	227.4	
NL4-3 D T V		R	>281.8	· ·
JRCSF G I V			2.1	٠.
JRCSF D I V			104.0	
	-			
a nimely of all 1 Viro	-1 72(2).986 -	.qqz		

Table 6

Entry Inhibitor Susceptibility: T-20 Fusion Inhibitor

Drug resistance mutations were introduced into well-characterized X4 tropic (NL4-3) and R5 tropic (JRCSF) viruses. T20 susceptibility was measured using the virus entry assay. The fold change (FC) in T-20 susceptibility for each virus was determined by dividing the IC50 of the test virus by the IC50 of the HXB2 strain of HIV-1. T-20 sensitivity of similar mutant viruses has been reported in the scientific literature (Rimsky et al.,).

b Fold change in IC50 (vs. HXB2) using PhenoSense HIV Entry Assay

4

Identifying Entry Inhibitor Resistance Mutations

Virus			AA	6	Sec	ene	u	Sequence ^a				
HXB2	0		7	8	9	\mathbf{I}	>	Q	Q	Q		
1	Q		_	2	9	Ι	>	Q	Q	Q		
2	Q		7	S		Н		Q	Q	Q		
3	Q			S	×	Ι	×	Q	Q	Q	X=G/S	X= ✓
4	Ò			8	9	H	>	Ò	Q	ð		
2	Ó			S	9	Η	>	Q	Q	Q		
9	Q			8	×	Η	×	Ŏ	Q	Q	X=G/S	X=
2	Q		7	8	9	Η	>	Q	Q	Q		
∞	Ò			8	9	Η	>	Ó	Q	Q		
6	Q		1	8	9	H	>	Q	Q	Q		
10	Q			8		Τ	>	Q	Q	Ò		
12	Q	1	_	8	×	I	>	Q	Q	Q	X=G/D	
13	ď			5	9	Η	>	Q	Q	Q		

a gp41 amino acid sequence positions 32 to 41



Identifyin	ng Entry Inhibitor Resistance Mutations
<u>Virus</u>	AA Sequence ^a
HXB2	QLLSGIVQQQ
1	QLLSGIVQQQ
2	QLLSSIMQQQ
3	Q L L S X I X Q Q Q $X=G/S$ $X=V/M$
4	QLLSGIVQQQ
5	QLLSGIVQQQ
• 6	QLLSXIXQQQX=G/SX=V/M
7	QLLSGIVQQQ
8	QLLSGIVQQQ
9	QLLSGIVQQ
10	QLLSDIVQQQ
12	QLLSXIVQQQ X=G/D
13	QLLSGIVQQQ

a gp41 amino acid sequence positions 32 to 41

Table 7

Identifying Envelope Protein Mutations.

Viruses with reduced (or increased) susceptibility to an entry inhibitor are identified using the virus entry assay. Mutations that may confer reduced (or increased) susceptibility to the entry inhibitor are identified by sequencing the envelope genes of the sensitive and resistant viruses. The deduced amino acid sequences of the sensitive and resistant viruses are compared to identify candidate drug resistance mutations. The ability of a specific mutation to confer altered drug susceptibility is confirmed or disproved by introducing the mutation into a drug sensitive virus and measuring the susceptibility of the mutant virus in the virus entry assay. In the example represented here, a short stretch of amino acid sequences within the first heptad repeat (HR-1) of the HIV-1 gp41 transmembrane envelope protein is aligned for viruses exhibiting different T-20 susceptibilities. Highlighted amino acids represent mutations known to confer reduced susceptibility to T-20.

Similar analyses can be used to identify envelope amino acid sequences that (a) alter/influence susceptibility to CCR5 or CXCR4 inhibitors, (b) specify X4, R5 and dual tropism, and © elicit neutralizing antibodies.